論 文 内 容 の 要 旨

応用生命化学　専　攻
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論文題目

Comprehensive Analysis of Coffee Bean Extracts by NMR Spectroscopy

（NMR によるコーヒー豆抽出物の非破壊分析）

Introduction

Coffee is one of the world's most widely consumed beverages. To investigate the chemical characteristics of coffee, lots of analytical techniques employing gas chromatography, liquid chromatography, UV–Visible spectrophotometry, mass spectrometry, or a combination of some of those methods, have been used. By these studies, hundreds of components have been detected from coffee beans, such as caffeine, trigonelline, proteins, amino acids, carbohydrates, carboxylic acids, chlorogenic acids, lipids, glycosides and minerals. However, all the above conventional methodologies are compound-targeted, which means only one or one kind of compounds can be considered in one observation, and require appropriate extraction, purification, and chemical derivatization of each component. It is reasonable to think that even a simple treatment could cause qualitative and quantitative modifications of the original mixture. Therefore, an un-biased, non-destructive, rapid but informative method of coffee analysis is still expected to be developed.

Modern nuclear magnetic resonance (NMR) spectroscopy with its dramatically improved resolution and sensitivity has been widely applied in food chemistry to identify organic compounds non-destructively and to structurally analyze biopolymers, as it is capable in a rapid, single experiment of simultaneously detecting multiple components without destruction of the food. By identifying the different spin systems from appropriate two-dimensional (2D) NMR spectra, comprehensive analysis with NMR makes it possible to identify and quantify the components of foods as complex mixtures without physically separating them. With an assigned spectrum, the comprehensive conditions of sample can be obtained just by a single observation with sufficient qualitative, quantitative and state-related information

Therefore, the purpose of the present study is to characterize coffee bean extracts by proton (^{1}H) and carbon (^{13}C) NMR spectroscopy. Detailed signal assignments of green and roasted coffee bean extracts were carried out using various 2D NMR spectra. On the basis of signal assignments, the qualitative, quantitative and state analyses of coffee bean extracts were accomplished. Furthermore, the chemical changes over time during coffee bean roasting process, as well as the discrimination of coffee beans according to their species and origins were performed by NMR spectroscopy.

Spectral Analysis of Green Coffee Bean Extracts

The quality of coffee is directly affected by the chemical composition of green coffee beans. Therefore, a complex mixture analysis by one-dimensional (1D) and 2D NMR spectroscopy was carried out for the identification and quantification of organic compounds in green coffee bean extract (GCBE). A combination of ^{1}H–^{1}H DQF-COSY, ^{1}H–^{13}C HSQC, and ^{1}H–^{13}C CT-HMBC sequences was used, and 16 compounds were identified. In particular, three isomers of caffeoylquinic acid (CQA) were identified in the complex mixture without any separation. In addition, GCBE components were quantified by the integration of carbon signals by use of a relaxation reagent and an inverse-gated decoupling method without a nuclear Overhauser effect. This NMR methodology provides detailed information about the kinds and amounts of GCBE components, and in this study, the chemical makeup of GCBE was clarified by the NMR results (*1*).

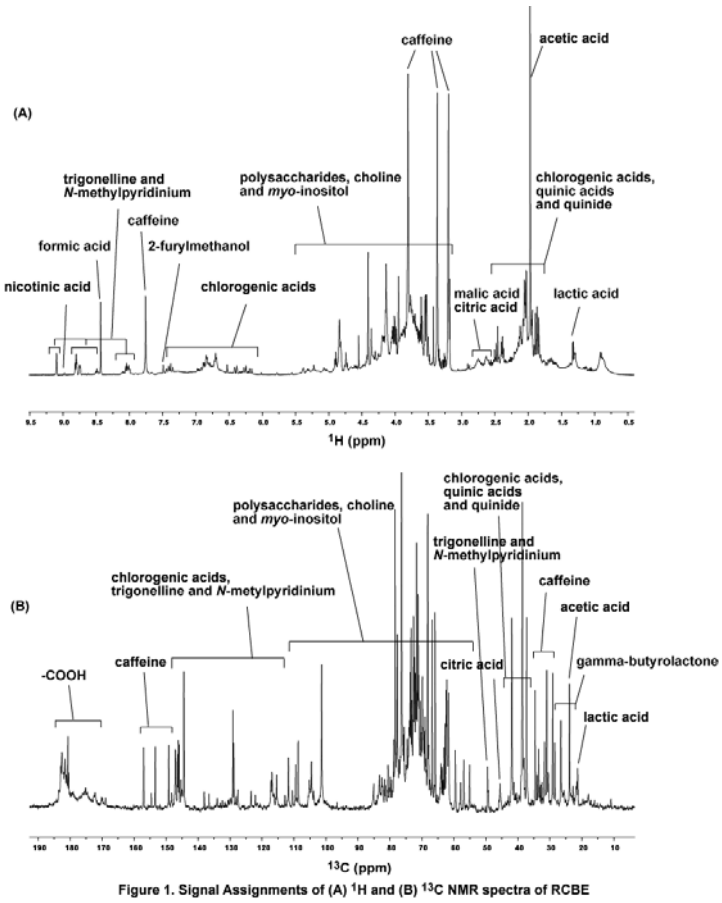

Figure 1. Signal Assignments of (A) ^{1}H and (B) ^{13}C NMR spectra of RCBE

Spectral Analysis of Roasted Coffee Bean Extracts

Roasted coffee bean extracts were characterized by ^{1}H, ^{13}C NMR spectroscopy. To identify the RCBE components, a detailed and approximately 90% signal assignment was carried out using various 2D NMR spectra including ^{1}H-^{1}H multiple-step Relayed COSY, ^{1}H-^{13}C Edited-HSQC, ^{1}H-^{13}C CT-HMBC and a spiking method. A total of 24 coffee components, including 5 polysaccharide units, 3 stereoisomers of chlorogenic acids, and 2 stereoisomers of quinic acids, were identified with the NMR spectra of RCBE as shown in **Figure 1**. On the basis of the signal integration, the coffee components were successfully quantified. At the same time, state analyses were further launched for the metal ion-citrate complexes, caffeine-chlorogenate complexes and interactions between high and low molecular weight components (*2*).

Chemical Changes of Coffee Bean Components during Roasting Process

The roasting process of green coffee beans is probably the most important factor in the development of flavors that let us enjoy a good cup of coffee. During the roasting process, the beans undergo many complex chemical reactions which are imposable to separate, leading to important physical changes and to the formation of the substances responsible for the sensory qualities of the beverage. In this part, a ^{1}H and ^{13}C NMR-based comprehensive analysis of coffee bean extracts at different degrees of roast were carried out. The roasting process of coffee bean extracts was chemically characterized using detailed signal assignment information coupled with multivariate statistical analysis. 30 NMR-visible components of coffee bean extracts were monitored simultaneously as a function of the roasting duration. As shown in **Figure 2**, during roasting, components such as chlorogenic acids **(A)** were degraded; on the other hand, components such as quinic acids **(B)** were generated, and some water-soluble polysaccharides **(C)** were generated and followed by degradation. Multivariate statistical analysis also indicated that some components, such as sucrose, chlorogenic acids, quinic acids, and polysaccharides, could serve as chemical markers during coffee bean roasting. The present composition-based quality analysis can offer an excellent holistic method and significant chemical markers to control and characterize the coffee roasting process (*3*).

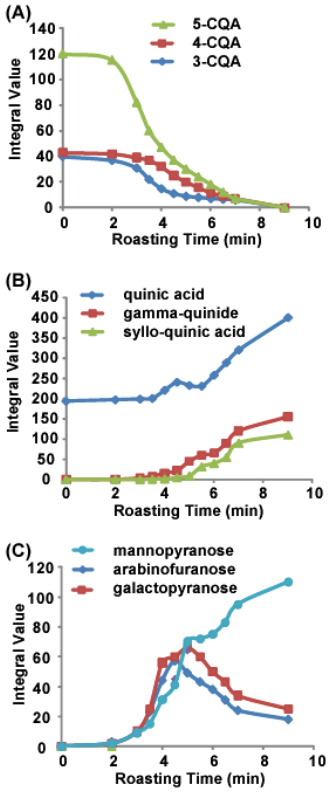

Figure 2. Evolusions of coffee components during roasting process. (A) chlorogenic acids; (B) quinic acids and quinids; (C) polysaccharides.

Discrimination of Coffee Beans according to Their Species and Origins

Coffee plant belongs to the *Rubiacea* family, of which over 500 genera and 6,000 species exist. But of the many species, only two are of economic importance, those belonging to *Coffea arabica* (arabica) and *Coffea cenephora*, or more commonly known as robusta. Arabica accounts for roughly 75% of total world production, while robusta the remaining 25%. Therefore, in the present study, ^{13}C NMR spectroscopy with assigning information, coupled with principal component analysis (PCA) and orthogonal partial least square discriminant analysis (OPLS-DA) models, were employed to distinguish the species and origins of green coffee beans, and identify the significantly different metabolites between species and origins. As shown in **Figure 3**, green coffee bean samples were distinguished by PCA according to their species **(A)** and origins **(B)**. The biomarkers such as sucrose, caffeine and chlorogenic acids were captured by OPLS-DA model (*4*).

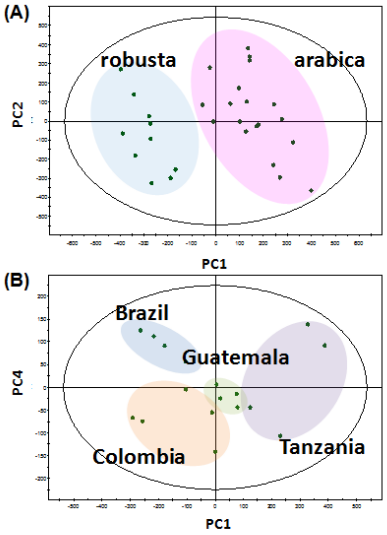

Figure 3. Discriminations of Green Coffee Beans by Their (A) Species and (B) Origins.

References

(1) Feifei Wei, Kazuo Furihata, Fangyu Hu, Takuya Miyakawa, and Masaru Tanokura. Complex Mixture Analysis of Organic Compounds in Green Coffee Bean Extract by Two-dimensional NMR Spectroscopy. *Magnetic Resonance in Chemistry*, **2010**, 48, 857-865.

(2) Feifei Wei, Kazuo Furihata, Fangyu Hu, Takuya Miyakawa, and Masaru Tanokura. Two-Dimensional ^{1}H-^{13}C Nuclear Magnetic Resonance (NMR)-Based Comprehensive Analysis of Roasted Coffee Bean Extract. *Journal of Agricultural and Food Chemistry*, **2011**, 59, 9065-9073.

(3) Feifei Wei, Kazuo Furihata, Masanori Koda, Fangyu Hu, Takuya Miyakawa, and Masaru Tanokura. Roasting Process of Coffee Bean as Studied by NMR: Time Course of Composition and Multivariate Statistical Analysis. *Journal of Agricultural and Food Chemistry*. (In revision).

(4) Feifei Wei, Kazuo Furihata, Masanori Koda, Fangyu Hu, Takuya Miyakawa, and Masaru Tanokura. ^{13}C NMR-Based Metabonomics for the Classification of Green Coffee Beans According to Varieties and Origins. (In preparation).